Jl S. Afr. Bot. 36 (1): 9–12 (1970)

SPIRODELA GROWTH TEST: A POSSIBLE BIOASSAY FOR ABSCISIC ACID*

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ABSTRACT

The growth response of *Spirodela oligorrhiza* in terms of dry weight production over a period of eight days is proportional to the $\log_{10}$ concentration of ABA and can be used as a sensitive, reliable and easily performed bioassay to detect amounts as low as $10^{-5}\mu g$. The assay is particularly reliable over the range 0·01 to 10,000μg.

UITTREKSEL

SPIRODELLA GROEITOETS: 'n MOONTLIKE BIOLOGIESE METING VIR AFSNYDINGSSUUR. Die groei reaksie van *Spirodela oligorrhiza* in terme van droë gewig produksie oor 'n tydperk van agt dae is eweredig met die $\log_{10}$ konsentrasie van ABA en kan gebruik word as 'n sensitiewe, betroubare en maklik uitvoerbare biologiese meting om hoeveelhede so min as 10^{-5} μg te bepaal. Die meting is veral betroubaar oor die reeks 0·01 tot 10,000 μg.

INTRODUCTION

Ohkuma, Smith, Lyon and Addicott (1963) reported on the use of the standard cotton explant bioassay to test the phenomenal abscission-accelerating properties of ABA. However, this bioassay, which employs the cotyledonary petioles of 14-day old cotton seedlings, also responds to ethylene and gibberellic acid. Milborrow (1967) described a bioassay in which the effect of ABA on coleoptile length in dissected wheat embryos was measured. He also developed a sophisticated racemate dilution method which, unfortunately, requires rather specialized equipment. Davis, Heinz and Addicott (1968) recently described a chemical assay using a gas-liquid chromatographic method to measure the trimethylsilyl derivatives of ABA.

The bioassay reported on here, involves the minimum of equipment; it is easily performed, but it does require a rather long culture period, namely eight days.

Accepted for publication 1st October, 1969.

* This research was supported by a grant from the Council for Scientific and Industrial Research, Pretoria, Republic of South Africa.

MATERIAL AND METHODS

Three sterile, two-frond plants of *Spirodela oligorrhiza* were selected for uniformity of stage of development and size, and transferred aseptically to each of 50ml Erlenmeyer flasks containing 30ml full strength Hoagland's nutrient medium with the appropriate dilution of ABA. Dilutions of ABA were made up with nutrient solution. Each flask was connected to a Swinnex adapter fitted with a GS $0 \cdot 22\mu$ filter disc and sterilized at $1 \cdot 05$kg/cm^2 for 15 minutes. The cultures were aerated for 45 minutes twice daily and were illuminated for 16 hours per day with fluorescent light at an intensity of ca. $3 \cdot 0 \times 10^3$ lm/m^2. Each experiment was terminated after either eight or twelve days, as preliminary experiments had shown that longer periods of time (e.g. up to 21 days) were unsuitable as they gave very variable results.

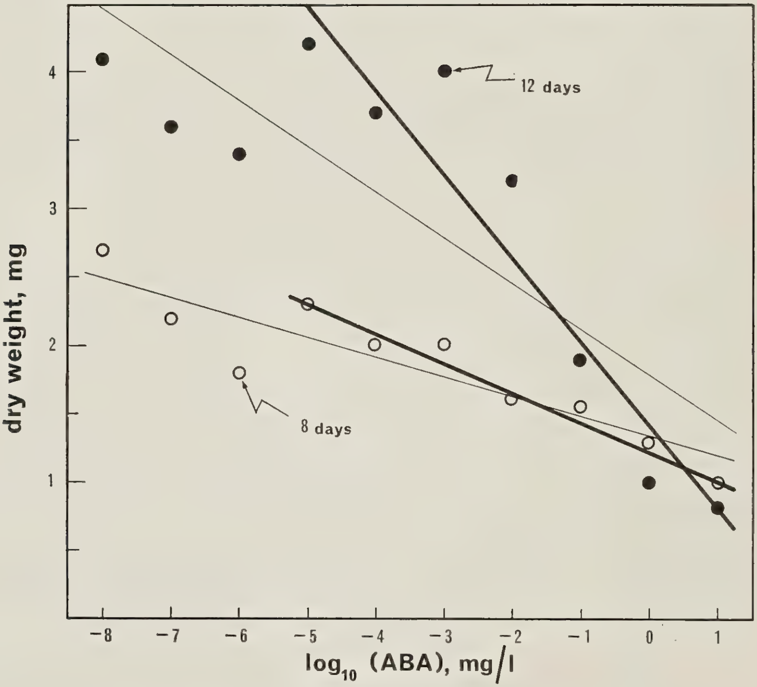

FIG. 1.
The relationship between the dry weight of *Spirodela* and the concentration of abscisic acid.

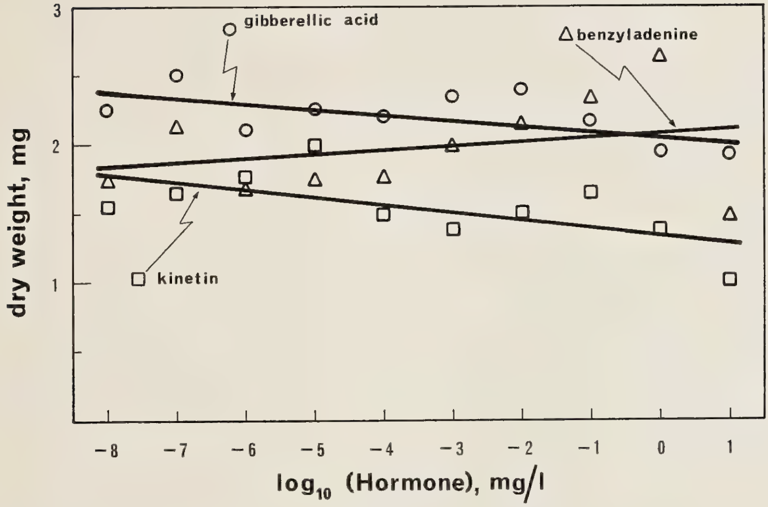

FIG. 2.
The relationship between the dry weight of *Spirodela* and the concentration of gibberellic acid, kinetin and benzyladenine.

The results reported in this paper are from six experiments with six three-plant replicates in each treatment. The same procedure was followed to determine the effects of kinetin, benzyladenine (BA) and gibberellic acid (GA) on the growth of *Spirodela*.

RESULTS AND DISCUSSION

The relationship between dry weight of *Spirodela* and concentration of abscisic acid for eight and twelve day growth periods are shown in Figure 1 and the relationship between dry weight and concentration of gibberellic acid, benzyladenine and kinetin are shown in Figure 2. For the eight day growth results the regression line fitted to the range of concentrations of ABA from 10^{-5} to 10mg/l gave a better fit than when a line was fitted to the whole range (10^{-8} to 10mg/l). The same applied to the results obtained for a twelve day growth period (Figure 1). A point to note is that the break between 10^{-6} and 10^{-5} mg/l of ABA has been obtained in repeated experiments and was reported earlier (Van Staden *et al.* 1969).

The usable range of this assay is therefore from 0·01 to 10,000μg, which is reasonable if it is taken into account that in the test of Davis *et al.* (1968) the lowest concentration of ABA detectable was 0·025μg.

The results of GA, BA and kinetin over the same range of concentrations are rather variable. It cannot be categorically stated at this time that their combined presence in an extract would not seriously affect the results of a bioassay on ABA. For our bioassay it would be necessary to use extracts which have been purified as much as possible, for example by employing the techniques of Rudnicki (1969) for extracting ABA from apple seed. In contrast Davis *et al.* (1968) were able to use extracted hormones with a minimum of preliminary purification. It must be pointed out that the present bioassay cannot be used for identifying ABA. Its identity has to be established by other methods, such as those of Milborrow (1967) and Rudnicki (1969).

The slope of the regression line for the relationship between concentration of ABA (10^{-5}—10mg/l) and dry weight of *Spirodela* over a twelve day growth period is greater than that for the eight day growth period, indicating a potentially more sensitive detection of ABA in a bioassay taken over a twelve day growth period than over an eight day period. The goodness of fit of the eight day results, however, was better than that for the twelve day results, indicating that an eight day period gives more reliable results.

Although, as previously reported (Van Staden *et al.* 1969) six plants per treatment appear the optimum number to work with, the results with three plants still gave satisfactory results. This saving in number of plants is important when selection criteria such as those used for a bioassay are imposed.

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